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Comparing interval and continuous exercise training regimens on neurotrophic factors in rat brain

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HIGHLIGHTS

- High intensity exercise training increases BDNF and GDNF in the brain.
- Interval training increases BDNF and GDNF in the brain more than continuous training.
- There is positive correlation between H_2O_2 and TNFα with BDNF and GDNF in the brain.

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ABSTRACT

The research literature suggests that oxidative stress and pro-inflammatory factors influence neurotrophins in vitro. However, there is insufficient information about their effects on exercise training conditions, especially during high intensity trainings. This study aimed to compare the effects of 6 weeks of high intensity interval and continuous training regimens on brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), hydrogen peroxide (H_2O_2), and tumor necrosis factor alpha (TNF-α) in the rat brain. For this purpose, twenty-four Albino Wistar rats were divided into sedentary control (SC), high intensity interval training (HIIT), and continuous training (CT) groups. Both HIIT and CT regimens increased H_2O_2 level and TNF-α concentration in the brain, and the alterations made were greater following HIIT than CT. In addition, both HIIT and CT regimens increased BDNF and GDNF concentrations significantly, with a higher elevation following HIIT than CT. Furthermore, H_2O_2 level and TNF-α concentration correlated positively with both BDNF and GDNF concentrations. Generally, high intensity interval training regimen, rather than continuous training regimen, is highly potential to improve BDNF and GDNF through a greater increase in H_2O_2 and TNF-α as oxidative stress and pro-inflammatory factors.

1. Introduction

Brain-derived neurotrophic factor (BDNF) and Glial cell line-derived neurotrophic factor (GDNF) are small peptides which belong to the family of neurotrophic factors and are distributed in different regions of central nerve system (CNS) [1]. BDNF modulates brain development and neuroplasticity and neurite outgrowth, thereby improving memory and preventing Alzheimer and depression [2–4]. Further, GDNF improves motor function by protecting dopaminergic, cortical, and motor neurons which in turn prevents the occurrence of Parkinson and Amyotrophic Lateral Sclerosis diseases [5–7]. However, the available evidence suggests that concentrations of BDNF and GDNF can be modulated by altered redox homeostasis [1,8,9], pro-inflammatory conditions [10–13], and exercise training [1,6,14,15].

In vitro, accumulating evidence has shown that the expression of BDNF and GDNF may be influenced by hydrogen peroxide (H_2O_2) [1,8,9] and tumor necrosis factor alpha (TNF-α) [10–13]. Nuclear factor-kappa B (NF-κB) and cAMP response element bind protein (CREB), as two main transcription factors, play important roles in the expression of BDNF and GDNF [9,11,12]. TNFα increases in the brain through microglia activation and peripheral TNF-α crossing from blood–brain barrier [11,16,17]. By interaction with its receptors, TNF-α induces neurotrophin expression not only through the activation of NF-κB but also via the activation of CREB [11,12]. In this context, it has been

Abbreviations: BDNF, Brain-derived neurotrophic factor; CNS, Central nerve system; CREB, cAMP response element bind protein; CT, Continuous training; GDNF, Glial cell line-derived neurotrophic factor; H_2O_2, Hydrogen peroxide; HIIT, High intensity interval training; IGF-1, Insulin-like growth factor-1; NF-κB, Nuclear factor-kappa B; SC, Sedentary control; TNF-α, Tumor necrosis factor alpha; VO_2max, Maximal oxygen uptake.

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shown that TNF-α induces BDNF and GDNF expression in astrocytes [12,13], trigeminal ganglion neurons [10], and dorsal root ganglia [18]. Besides, exogenous and astrocytes-released TNF-α induces nerve growth factor and GDNF production in astrocytes [13]. However, anti-TNF-α treatment inhibits BDNF release from astrocytes [12]. Similarly, H_2O_2 upregulates BDNF and GDNF expression by increasing translocation of p65:p50 of NF-κB complex from cytoplasm to nucleus [1,19–21]. Also, the activation of NADPH oxidase (as a stress oxidative resource) increases BDNF expression by enhancing CREB phosphorylation [9]. In this context, it has been indicated that H_2O_2 injection to rats increases GDNF protein concentration in cervical spinal cord region [1]. Another study has pointed out that H_2O_2 increases GDNF protein in neuron-glia mixed cultures and protects dopaminergic neurons [21]. However, it has been shown that antioxidant N-acetyl-L-cysteine inhibits BDNF release from the microvascular endothelial cell line of the brain [8], and N-tert-butyl-α-phenylnitrone reduces BDNF protein of the cervical spinal cord in rats [1].

Identifying the factors which regulate the brain BDNF and GDNF availability is an important goal for brain health and function [22]. Exercise training accounts for a non-pharmacological approach to modulating neurotrophins [4]. It is maintained that chronic swimming exercise [3], treadmill running [1,5,6], and wheel running [22–24] elevated neurotrophins in different regions of the nerve system of young [15], adolescent, and old animals [7,25]. In addition, it has been shown that low to moderate exercise training improves memory function and balance coordination through increasing BDNF and GDNF concentrations in hippocampus [3] and striatum [5], respectively. Furthermore, daily and alternating days of wheel running increase BDNF concentration in hippocampus [22]. BDNF protein elevates 2 weeks after the exercise ends, while it progressively declines following 4 weeks of detraining [24]. Also, it has been shown that long-term running at moderate intensity [5,23], contrary to short-term running [26], increased GDNF concentration in the striatum and sciatic nerve.

Although low to moderate exercise training regimens increase BDNF and GDNF concentrations in different regions of the brain [3,5,22,23], effects of intensive exercise trainings on BDNF and GDNF have not yet been well established. The importance of this issue becomes even more evident since we know that intensive exercise trainings increase H_2O_2 [27] and TNF-α production [28]. In reality, interactive effects of H_2O_2 and TNF-α on neurotrophin adaptations induced by intensive exercise training have not yet been sufficiently investigated. Furthermore, it seems that intensive interval and continuous training regimens may activate stress oxidative resources [29] and antioxidant system differently [30], and thereby produce different levels of H_2O_2. Therefore, it is proposed that neurotrophin adaptations may be influenced differently by intensive interval and continuous exercise training regimens. Many people do not have enough time for exercise, and it is necessary to examine the effects of exercise training intensity on health improvement, especially neurotrophin adaptations. Hence, the aim of the present study was to compare the effects of high intensity interval and continuous training regimens on BDNF and GDNF concentrations in the rat brain and determine their relations with H_2O_2 and TNF-α as two possible modulating mechanisms.

2. Materials and methods

2.1. Animals

All animal experiments conformed to the guidelines for the use and care of laboratory animals ("Principles of laboratory animal care", NIH publication No. 86-23. Revised 1996), and the study was approved by the ethics committee of Birjand University of Medical Sciences in Iran. Twenty-four mature male Albino Wistar rats (3 months old) with a weight equal to 282 ± 14 g were prepared from the laboratory of bearing and multiplying at the Mashhad University of Medical Sciences in Iran. The rats were housed in standard cages of polycarbonate (20 × 59 × 38 cm) in a room at a temperature of 22 ± 2 °C with a 12:12-h reverse light–dark cycle starting the light period at 7:00 am. The rats had free access to tap water and standard rat food (Javaneh Khorasan Company, Iran). All the animals were checked daily for clinical signs of diseases. The animals were randomly divided into three equal groups (n = 8) of sedentary control (SC), high intensity interval training (HIIT), and continuous training (CT).

2.2. Exercise training protocols

Exercise training was performed on a 12-lane treadmill because the intensity and duration of exercise could be controlled easily [15]. The animals were familiarized with running on a motor-driven treadmill (5 days, 10 min/day at a speed of 10 m/min) [31]. Continuous and interval exercise trainings were performed on the basis of overload principle for 6 weeks, 6 sessions per week (Table 1) [32]. Overload was exerted by increasing time and intervals in CT and HIIT groups, respectively. At the beginning and end of continuous and interval exercise training regimens, warm-up and cool-down were performed at 16 m/min. This intensity corresponds to 68% maximal oxygen uptake (VO_2max). Besides, intensities of continuous and interval exercise training regimens correspond to 80 and 95–100% VO_2max, respectively. Active rest was performed between intervals in HIIT group for 60 s at 16 m/min [32]. The rats were motivated to run via the electrical shocks at the rear of the treadmill and by gentle prodding using a sponge [1]. Each animal was assigned to a fixed lane and all the activities were performed in the respective lane during the entire training program to minimize novelty confound [26]. The rats of the SC group were transported daily to the training room, exposed to the same environment as the exercising groups, and placed on the treadmill without running for as long as the exercising groups were on the treadmill [5].

2.3. Tissue preparation

To avoid data misinterpretation due to the remaining effects of the last exercise session, the rats were sacrificed by decapitation under deep anesthesia (Ketamine, 60–80 mg/kg and Xylazine, 8 mg/kg; IP) 48 h after the last exercise session [5] between 10:00 and 11:00 am. The whole brain of each rat was removed and dissected in less than 5 min and washed by normal saline to remove excess surface blood. The brain was rapidly submerged in liquid nitrogen for 2 min and finally stored at −80 °C for further analysis.

2.4. Biochemical assays

In order to measure all the parameters from the same region and because of different distributions of GDNF [20] and BDNF [33] in the brain, we selected the whole brain for biochemical assays [2]. Each brain was dipped in liquid nitrogen and smashed into a fine powder [7,34]. For total proteins of GDNF, BDNF and TNF-α assays, we added 1 ml 1× Phosphate Buffered Saline and Protease Inhibitor Cocktail (#GB-326-1, ProBlock™-50, Goldbio technology CO, USA) to the microtube containing 52–87 mg powdered brain tissue and stored them overnight at −20 °C. After two freeze–thaw cycles which were performed to break the cell membranes, the homogenate was vortexed (Stuart Mixers Vortex, SA8™, England) and centrifuged (Eppendorf Centrifuge, Mini Spin^R, Germany) for 5 min at 5000 ×g, at 2–8 °C. For H_2O_2 assay, after the addition of 400 μl of 1× Phosphate Buffered Saline to 54–97 mg of powdered brain tissue and performing vortex, the sample was centrifuged for 15 min at 7000 rpm. The supernatant was removed and the assays were carried out immediately according to the manufacturer's instructions. We used the commercially 96-well ELISA kits to measure the protein contents of total GDNF (#CSB-E04566r, Cusabio Biotech CO., LTD. Sino-American), total BDNF (#CSB-E04504r, Cusabio Biotech CO., LTD. Sino-American) and total TNF-α (#865.000.096, Diaclone SAS., France) in the brain. The sensitivities of the kits were less than 7.81,

Table 1
Intensive interval and continuous exercise trainings protocols.

Week	Day	CT	HIIT	
			Odd day	Even day
Week 1	1	20 min, 27 m/min	2 intervals, 40 m/min, 3 min	
	2	22 min, 27 m/min		3 intervals, 54 m/min, 30 s
	3	24 min, 27 m/min	2 intervals, 40 m/min, 3 min	
	4	26 min, 27 m/min		5 intervals, 54 m/min, 30 s
	5	28 min, 27 m/min	2 intervals, 40 m/min, 3 min	
	6	30 min, 27 m/min		7 intervals, 54 m/min, 30 s
Weak 2	1	32 min, 27 m/min	3 intervals, 40 m/min, 3 min	
	2	34 min, 27 m/min		9 intervals, 54 m/min, 30 s
	3	36 min, 27 m/min	3 intervals, 40 m/min, 3 min	
	4	38 min, 27 m/min		11 intervals, 54 m/min, 30 s
	5	40 min, 27 m/min	3 intervals, 40 m/min, 3 min	
	6	42 min, 27 m/min		13 intervals, 54 m/min, 30 s
Weak 3	1	44 min, 27 m/min	4 intervals, 40 m/min, 3 min	
	2	46 min, 27 m/min		15 intervals, 54 m/min, 30 s
	3	48 min, 27 m/min	4 intervals, 40 m/min, 3 min	
	4	50 min, 27 m/min		17 intervals, 54 m/min, 30 s
	5	52 min, 27 m/min	5 intervals, 40 m/min, 3 min	
	6	54 min, 27 m/min		19 intervals, 54 m/min, 30 s
Weak 4	1	56 min, 27 m/min	5 intervals, 40 m/min, 3 min	
	2	58 min, 27 m/min		19 intervals, 54 m/min, 30 s
	3	60 min, 27 m/min	6 intervals, 40 m/min, 3 min	
	4	60 min, 27 m/min		20 intervals, 54 m/min, 30 s
	5	60 min, 27 m/min	6 intervals, 40 m/min, 3 min	
	6	60 min, 27 m/min		20 intervals, 54 m/min, 30 s
Weak 5–6	1–12	60 min, 27 m/min, to end of 6th week	6 intervals, 40 m/min, 3 min, to end of 6th week	20 intervals, 54 m/min, 30 s, to end of 6th week

CT, continuous training; HIIT, high intensity interval training.

7.81, and 15 pg/ml for BDNF, GDNF and TNF-α, respectively. Brain H_2O_2 assay was carried out by 96-well colorimetric assay kit (#BC05-96, Biocore Diagnostik Ulm, German) with 10 μM assay sensitivity. The absorbance of GDNF, BDNF and TNF-α was measured at 450 nm and H_2O_2 at 546 nm by Anthos 2020 microplate reader (Biochrom CO, England), and the contents were expressed in mg tissue weight.

2.5. Statistical analysis

The collected data were analyzed in SPSS software (version 16.0) and presented as means ± SD. Initially, Shapiro–Wilk's and Levene's tests were performed on all dependent variables to test normality and equality of variances, respectively. Statistical significance was determined at $P < 0.05$ using one-way analysis of variance followed by Bonferroni post-hoc comparison to test the differences between groups. In addition, Pearson's correlation coefficient was calculated to determine the relationship between variables.

3. Results

There was no significant difference between the animals' body weight in HIIT (332 ± 4 g), CT (338 ± 16 g) and SC (343 ± 9 g) groups at the end of the protocols ($F_{2,23} = 2.11$, $P = 0.14$) (Fig. 1).

The results indicated that the brain H_2O_2 level, as a marker of oxidative stress, increased significantly in HIIT (1.03 ± 0.16 μM/mg tissue) ($P = 0.001$) and CT (0.81 ± 0.16 μM/mg tissue) ($P = 0.01$) groups more than in SC (0.59 ± 0.10 μM/mg tissue) group, while the HIIT resulted in a greater increase in the level of brain H_2O_2 than those of CT ($P = 0.02$) (Fig. 2A). In addition, brain TNF-α, as a pro-inflammatory marker, increased significantly in HIIT (3.72 ± 0.27 pg/mg tissue) ($P = 0.001$) and CT (2.82 ± 0.36 pg/mg tissue) ($P = 0.001$) groups than in SC (1.53 ± 0.24 pg/mg tissue) group. However, the HIIT resulted in a greater increase in the concentration of brain TNF-α than CT ($P = 0.001$) (Fig. 2B).

In the context of neurotrophins, brain BDNF increased significantly in HIIT (33.79 ± 2.23 pg/mg tissue) ($P = 0.001$) and CT (24.02 ± 4.27 pg/mg tissue) ($P = 0.001$) groups than in SC (13.58 ± 1.46 pg/mg tissue) group. In addition, brain GDNF increased significantly in HIIT (26.64 ± 2 pg/mg tissue) ($P = 0.001$) and CT (17.1 ± 3.33 pg/mg tissue) ($P = 0.001$) groups than in SC (9.87 ± 1.27 pg/mg tissue) group. Besides, HIIT induced a greater increases in BDNF ($P = 0.001$) (Fig. 2C) and GDNF ($P = 0.001$) (Fig. 2D) of the brain than CT.

Furthermore, our results showed a significantly positive correlation between BDNF and H_2O_2 ($r = 0.88$, $P = 0.001$) (Fig. 3A), BDNF and TNF-α ($R = 0.89$, $P = 0.001$) (Fig. 3B) as well as between GDNF and H_2O_2 ($r = 0.89$, $P = 0.001$) (Fig. 3C), and GDNF and TNF-α ($r = 0.85$, $P = 0.001$) (Fig. 3D).

4. Discussion

In recent years, there has been a growing interest in evaluating the effects of exercise training on various neurological factors that improve cognitive processes and increase resistance to brain injury [1,4,28,35]. Exercise training activates many signal transduction pathways involved in neuroprotection in the brain through H_2O_2 [1] and TNF-α [28,36] production. Here, in an experimental animal model, it was revealed that HIIT and CT resulted in significant increases in H_2O_2 level and TNF-α, BDNF and GDNF concentrations in Albino Wistar rats. In addition, we

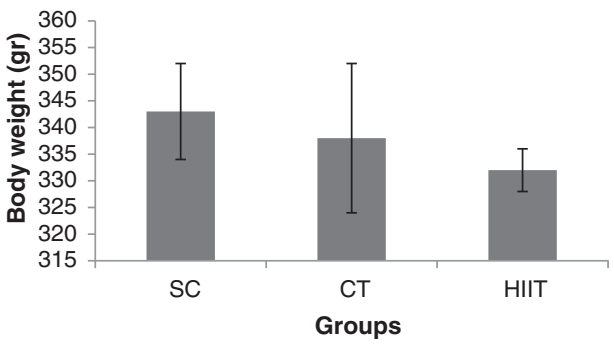

Fig. 1. Animals' body weight in high intensity interval training (HIIT), continuous training (CT) and sedentary control (SC) groups at the end of the protocol. No significant difference between the groups ($P = 0.14$).

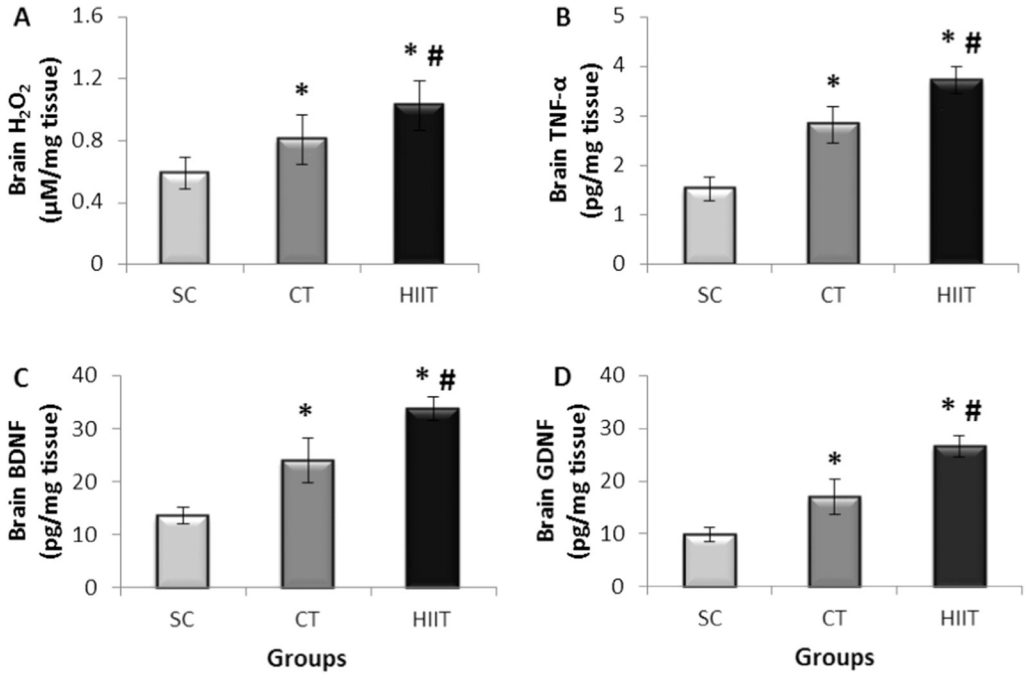

Fig. 2. Comparisons of H_2O_2 level (*P = 0.01 and P = 0.001 for CT and HIIT respectively, #P = 0.02) (A), TNF-α concentration (*P = 0.001, #P = 0.001) (B), BDNF and GDNF concentration (*P = 0.001, #P = 0.001) (C and D, respectively) between HIIT, CT and SC groups. The asterisk (*) indicates a significant difference from SC. The hash sign (#) indicates a significant difference from CT. Abbreviations are the same as are denoted in the legend of Fig. 1.

observed significant positive relationships between H_2O_2 and TNF-α, as oxidative stress and pre-inflammatory markers, and BDNF and GDNF, as neurotrophic factors.

There is now a substantial body of evidence suggesting that exercise training with low intensity is a significant factor against neurological diseases and that it influences memory [3] and motor functions [5] through increasing neurotrophic factors. GDNF with 15 kDa synthesizes in substantia nigra, releases to the corpus striatum, and protects dopaminergic, cortical, and motor neurons [1,20]. Our findings showed that brain GDNF increased following interval and continuous training at high intensity. Although the intensity of exercise training is a main factor in increasing GDNF in slow- [37] and fast-twitch [34] myofibers, it does not seem to be the single parameter in regulating GDNF in CNS. Previous studies have shown that long-term exercise trainings at moderate intensity increases GDNF concentration in striatum, sciatic nerve and spinal cord of C57BL/6 mice [5,23], while short-term exercise training at low to moderate intensity does not influence the GDNF concentration in the striatum and substantia nigra [26]. BDNF, as the most abundant neurotrophins with 14 kDa, is widely distributed in the CNS and associated with memory and learning processes [1,2]. Results of

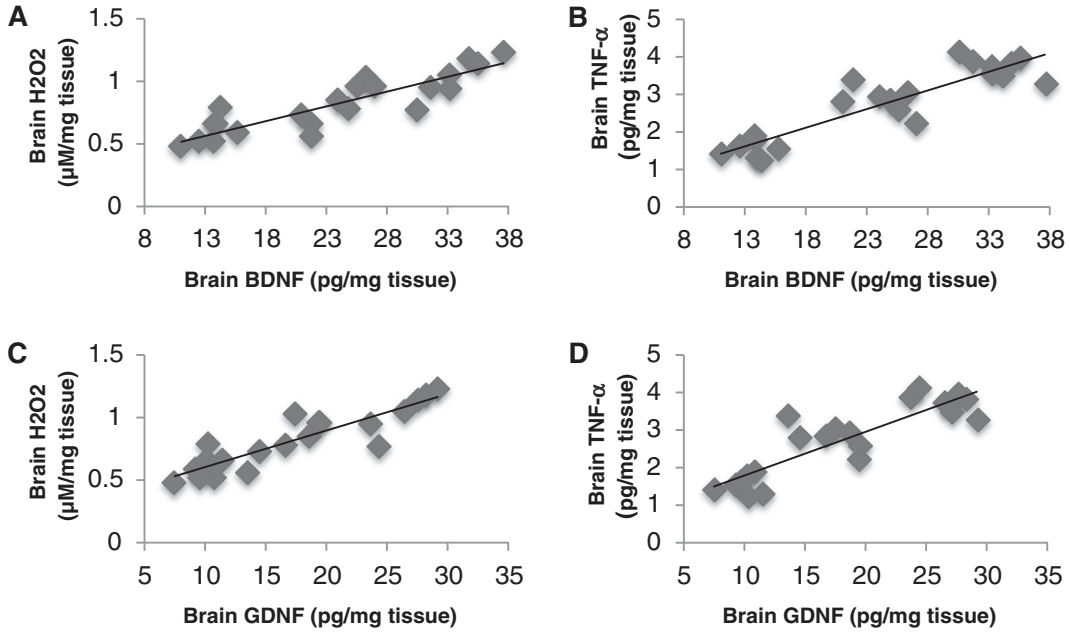

Fig. 3. Correlations between BDNF and H_2O_2 (P = 0.001) (A), BDNF and TNF-α (P = 0.001) (B), and GDNF and H_2O_2 (P = 0.001) (C) and GDNF and TNF-α (P = 0.001) (D) in the rat brain.

the present study demonstrate that both HIIT and CT increased brain BDNF. Our findings are inconsistent with other studies because of their short-term exercise training period (3, 7, 15 days running on treadmill) [38] and learning induced by Morris maze task at the last week of the exercise training [39] that resulted in an equal BDNF in the hippocampus and basal forebrain in both control and exercise training groups, respectively. In this regard, a significant positive correlation has been reported between BDNF protein and performance in the radial water maze [24]. Because of learning effects on BDNF, we tried to minimize any novelty learning during the entire training program. Running is a relatively simple motor task and easily learned [40]. Therefore, it was not necessary to familiarize rats with running on treadmill with high intensity and long duration that may be interfering with the results induced by exercise trainings. In reality, observed changes in BDNF concentration in this study may be due to the exercise training itself. In two studies, increased BDNF in hippocampus of female rats has been attributed to increased levels of estrogen and its receptors following long-term running at low to moderate intensity on treadmill [25] and wheels running [41]. Also, exercise training increased brain uptake of circulating insulin-like growth factor-1 (IGF-1) [35] and increased BDNF concentration in the hippocampus has been attributed to increased IGF-1 following short-term moderate intensity exercise training [6]. Intriguingly, it has been reported that both high [2] and low [25] intensity exercise trainings enhance BDNF concentration in the brain due to the attenuation of corticosterone concentration. Besides, three-month endurance training increased the release of BDNF protein in overweight subjects because of leptin reduction [14]. Finally, Berchtold and colleagues have reported that 28 days of daily and interval voluntary wheel running increased BDNF concentration in the hippocampus as a result of increasing the conversion of pro-BDNF to the mature form [22]. In the present study, BDNF and GDNF concentrations were measured in all brain regions. Since, a variety of cells including neurons, oligodendrocytes, glia cells, astroglia, microglia, satellite cells, schwann cells, stem cells and blood cells are involved in releasing BDNF and GDNF, we cannot attribute these changes to specific types of cells as it is suggested by other authors too [2,7].

Generally, the increased conversion of pro-BDNF to the mature form [22], IGF-1 [6,14] and estrogen [25,41], as well as reduced leptin [14] and corticosterone concentrations [25] has been considered as possible mechanisms for an increase in neurotrophins following low to moderate intensity exercise training. Exercise protocols in the present study were intense (with 80–100% VO_2max) resulting in increased H_2O_2 and TNF-α concentrations. In contrast, other studies have reported no significant changes in the production of H_2O_2 in the hippocampus and cerebellum and TNF-α content in the whole brain after low intensity [27] and short duration of exercise training [36], respectively. However, it has been reported that intensive exercise training at 80% VO_2max increases brain resistance to ischemic by increasing TNF-α concentration in healthy rats' brain [28]. On the other hand, Cotman et al., in a review, have pointed out to anti-inflammatory aspects of exercise training in pathological models [4]. Overexpression of TNF-α occurs during acute pathological and chronic exercise conditions such that its chronic overexpression is associated with neuroprotection [28]. Compared with continuous training, interval training may lead to further activation of the electron transport chain [32], shear stress on endothelial cells, and subsequently more activation of NADPH oxidase [29,42]. These may reflect higher H_2O_2 level induced by the type of exercise training. Furthermore, TNF-α high concentration in the rat brain following high-intensity exercise has been attributed to the reduced in interleukin-6 and -10 [15] and increased proportion and activation of astrocytes [17] and microglia [16] in the brain. Using a variety of methods, it has been shown that H_2O_2 and TNF-α result in the translocation of p65:p50 of NF-κB complex from cytoplasm to nucleus and binding it to the target sites in the DNA, thereby inducing BDNF and GDNF expression [1,11,12,19–21]. It is believed that oxidative stress and pro-inflammatory factors phosphorylate CREB and increase BDNF expression [9,12] as well. We found significant positive correlations between H_2O_2 and TNF-α with BDNF and GDNF concentrations. These findings are consistent with another study in terms of the significant relationship between free radicals and BDNF concentration in rat spinal cord [1]. Also, HIIT resulted in greater increases in BDNF and GDNF concentrations in the brain than CT, and these differences may be related to the higher concentrations of H_2O_2 and TNF-α in the brain after HIIT. Increased hippocampus TNFα and BDNF concentrations following intensive exercise training have been recently reported in adolescent rats [15]. BDNF is released by the cerebral vascular endothelium following hypoxic stresses [8], and it seems that stress oxidative induced by hypoxic condition during intensive exercise training has the potential to increase BDNF concentration in the brain [40]. Also, chronic inflammation occurs during intensive exercise, and BDNF elevation during exercise training has been attributed to inflammation [2]. Neurotrophins, oxidative stress, and pro-inflammatory markers showed similar increases after both interval and continuous exercise trainings. These pieces of evidence suggest that changes in brain BDNF and GDNF may be associated with enhanced oxidative stress and pro-inflammatory factors following intensive exercises.

5. Conclusions

Although numerous and direct evidence is not available in support of this notion, it appears that the HIIT, through further increases in H_2O_2 and TNF-α, can result in greater improvements in the BDNF and GDNF concentrations of rat brains. This means that the separation of the training sessions to various bouts of exercise with maximum effort (in the form of interval exercise) will lead to greater neurotrophin gains. The extent to which H_2O_2 and TNF-α can influence neurotrophins in the brain has remained ambiguous. Indeed, additional studies, through the use of knock-out rat, blocking antibodies, antioxidant parameters, and anti-inflammatory/antioxidant supplementations are required to obtain better perspectives about the effects of H_2O_2 and TNF-α on neurotrophins adaptations induced by exercise training.

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